# THE "DELFT SCHOOL" AND THE RISE OF GENERAL MICROBIOLOGY[1]

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Antonie van Leeuwenhoek certainly started something when he began making his lenses and examining anything he could lay his hands on! His observations led, about 300 years ago, to the discovery of the "little animals", now known under the names of protozoa and bacteria. And thus Leeuwenhoek, the Delft draper and scientist, became the "Father of protozoology and bacteriology," as Dobell (1) has so aptly called him. There is no doubt that the science of general microbiology began in Delft.

It was an exciting beginning. The animalcules were found almost everywhere, and they appeared to represent an astonishing array of sizes and shapes. Practically any kind of material revealed their presence—a wonder to behold, a pleasure to watch. And Leeuwenhoek made the most of his discovery: witness the enormous number of letters which he sent to the Royal Society of London vividly describing his observations with many pertinent reflections upon their significance.

Yet we do not ordinarily think of Leeuwenhoek as the founder of a "Delft School", or, for that matter, of any school. He was a solitary worker, and occasionally even reluctant to disclose to others the methods he employed. In consequence we are, even today, confined to speculation when it comes to deciding whether or not Leeuwenhoek might have hit upon a way of examining specimens by using some sort of dark-field illumination, a possibility that was suggested by Dobell (1, p. 331–2). This suggestion has been more fully discussed by Cohen (2) and Kingma Boltjes (3).

Now, Leeuwenhoek did not start a "school," and so his methodology was handed down to posterity only insofar as his letters divulged. There were no pupils who might afterwards have revealed secrets which the master had decided not to publish. But his discoveries were so spectacular and so unexpected that they could not fail to fire the imagination of others, equally imbued with curiosity, that driving force of scientific endeavor. Hence his observations were, in the course of time, repeated and extended in other places, and knowledge concerning the microbes gradually accumulated, until today there is available an amount of information so vast that it would be impossible for a single individual to be conversant with more than a small part of it.

This is an unfortunate although inevitable result of expanding scientific activity: the interested individual must needs make a choice as to what shall occupy his mind and hands. Thus there is a real danger that he may become involved in minutiae; a narrow specialist who "knows more and more about less

[1] Based on the A. J. Kluyver Lecture delivered before the Society of American Bacteriologists. Cincinnati, Ohio, May 19, 1949.

and less." However, an increase in factual information, the only solid basis for scientific accomplishment, also brings with it the desire for organizing and integrating the details. If this is satisfactorily accomplished the isolated data can be connected together into a framework composed of general principles. And the latter mark the culminating advances of science.

In what follows I shall try to sketch the gradual development of some principles in the realm of general microbiology; to show how these are associated with a "Delft School"; and to indicate how they have contributed to the rapidly growing interest in this field.

Obviously, Leeuwenhoek's discovery of the existence of the "little animals" raised problems concerning their origin, their activities, and the significance of the latter. Leeuwenhoek himself expressed opinions on these questions that were essentially identical with those which, two centuries later, became the established scientific views. During the intervening years the issues were, however, ardently debated on the basis of seemingly conflicting experimental results, and these experiments have added greatly to our store of knowledge.

I shall not dwell upon the fascinating controversy about the spontaneous generation of the microbes *versus* their origin from preexisting ones. The battle, earlier fought over the origin of larger organisms, and quite recently again over that of viruses, ultimately led to an acknowledged victory of the proponents of the idea of biogenesis. In the meantime the discrepancies in the outcome of many crucial experiments gradually led to the development of an adequate methodology for the study of microörganisms. Most of the techniques now so confidently used represent modifications and refinements of methods that had once produced results interpreted in favor of spontaneous generation. Thus can the mistakes made in scientific investigations be turned to advantage, for they lead to the recognition of unexpected and unpredictable sources of error, and so permit the eventual elimination of the latter.

But the apparent defeat of the doctrine of spontaneous generation left unsolved the fundamental problem of the origin of life. In recent years new ideas have been expressed, notably by Haldane (4), Oparin (5), and Horowitz (6) which have a strong scientific appeal because they suggest a way out of what otherwise would be an impasse. Whether these concepts can soon be made the basis for a renewed experimental attack cannot now be decided; the answer must be left to future studies.

So much for the first problem. The question of the activities of the "little animals," too, was contemplated by Leeuwenhoek, and once again he reached a conclusion that was not to become part of our scientific outlook until two centuries later. I do not here refer to the concept that microörganisms can play a role as causative agents of disease, but to the far broader one concerning their function in the cycle of matter. It should be realized that the former activity represents no more than a very minor aspect of this general phenomenon.

The important part played by microörganisms in transforming organic and inorganic substances on earth with the result that these may be used over and over again to sustain life of other organisms was first clearly expressed by Ferdinand Cohn (7) in 1872. In thus making possible the continuation of the great experiment of evolution the "little animals" occasionally perform their task in a manner that clashes with the desires of man who, through ignorance and greed, has a propensity for eliminating various natural resources from participation in the natural cycle of matter, and often appears to regard the earth with all that is on it as his own private property. This has led to an unwarranted emphasis on such resented activities of the microbes as would interfere with man's hoarding instinct, even to the point of making him lose sight of the fundamental significance of an uninterrupted continuation of the cycle. Those who have learned to view life in a wider sense can but hope that, through education, a better comprehension may gradually be reached, and the hoarding instinct be curbed—if there is still time.

Our knowledge of the diverse types of microbes responsible for the specific major transformations of matter has advanced greatly since Cohn's pronouncement. The most important contributions to this problem we owe to M. W. Beijerinck (8), the second of the great Delft microbiologists. By introducing the principle of enrichment cultures he opened the way for a rational approach to microbial ecology. Although some of Beijerinck's specific discoveries are fairly well-known to most microbiologists, the fundamental ideas that led to them have been appreciated far too little. This, I believe, is due to the fact that Beijerinck, who could have written a treatise on enrichment cultures that would not have failed to exert a profound influence, never so much as published a paper in which the principle was clearly formulated and its potentialities developed. When, in 1905, Beijerinck was awarded the Leeuwenhoek medal by the Koninklijke Akademie van Wetenschappen in Amsterdam, F.A.F.C. Went noted the above mentioned deficiency in his presentation address with the following words:

"There is in your publications such a wealth of original concepts and of special approaches, often buried in a couple of sentences, that such a treatise would surely be anticipated with the utmost interest. It would then also become clear how many of the current ideas in microbiology we really owe to you; this is far more than is apparent to those who merely have taken superficial notice of your publications" (9).

It was only on this occasion that Beijerinck stated his objectives and approach. I translate from his acceptance speech:

"I am happy to note that the way in which I approach microbiology has the approval of the best judges. This approach can be concisely stated as the study of microbial ecology, *i.e.*, of the relation between environmental conditions and the special forms of life corresponding to them. It is my conviction that, in our present state of understanding, this is the most necessary and fruitful direction to guide us in organizing our knowledge of that part of nature which deals with the lowest limits of the organic world, and which constantly keeps before our mind the profound problem of the origin of life itself. Therefore it is a great satisfaction to me that the Academy apparently wishes to honor the experimenter who exploits this field.

"In an experimental sense the ecological approach to microbiology consists of two complementary phases which give rise to an endless number of experiments. On the one hand it leads to investigating the conditions for the development of organisms that have for some reason or other, perhaps fortuitously, come to our attention; on the other hand to the discovery of living organisms that appear under predetermined conditions, either because they alone can develop, or because they are the more fit and win out over their competitors. Especially this latter method, in reality nothing but the broadest application of the elective culture method, is fruitful and truly scientific, and it is no exaggeration to claim that the rapid and surprising advances in general microbiology are due to this methodology. Nevertheless, and this in spite of the fact that Leeuwenhoek, more than two hundred years ago, already used this aspect of micro-ecology in some of his studies, and that Pasteur was enabled to make most of his great discoveries because he was guided by the same principle, the number of conscious exponents has so far remained very small. And I feel that I certainly may be reckoned among them because of the enthusiasm that is in me to contribute to the grand task that can here be accomplished" (10).

That is all. And who would bother to read these sentences, representing half a page of a printed speech, and written, like Leeuwenhoek's letters, in the Dutch language, some 45 years ago? Beijerinck never got around to writing the treatise Went had suggested, probably because he was more interested in doing experiments, and so the number of workers who consciously applied Beijerinck's principles remained small, limited, in fact, to those who had the good fortune of experiencing his influence, directly or indirectly. It is true that in 1907 Stockhausen (11) published a number of essays on microbial ecology ("Enrichment cultures after Beijerinck") in the "Wochenschrift für Brauerei," also issued in book form. But thirty years after its publication the first edition of the book was still far from exhausted, and many microbiologists have probably never heard of it.

Nonetheless, the fundamental significance of Beijerinck's work is slowly becoming recognized in wider circles, and the application of enrichment culture practices is spreading. Even such up-to-date studies as those concerned with the search for antibiotics, with the attempts to culture various algae and protozoa, an endeavor so successfully pursued by Pringsheim (12), and with the selection of specific nutritional types of microbes; all such studies are now generally carried out with the conscious or unconscious inclusion of Beijerinck's principles. Furthermore, if one thinks about the reasons for the ready availability of cultures of nearly all kinds of microörganisms (yeasts, algae, actinomycetes, sulfur and hydrogen bacteria, species of *Acetobacter*, *Azotobacter*, *Aerobacter*, *Spirillum*, *Mycobacterium*, *Propionibacterium*, or *Clostridium*, lactic acid bacteria, bacteria decomposing cellulose, agar, or urea, denitrifying and sulfate-reducing bacteria, methane-producing, luminous, or photosynthetic bacteria) it becomes abundantly evident that these reasons are not to be found primarily in the existence of pure culture collections, however useful a purpose they may serve, but chiefly in the simple methodology, based on Beijerinck's enrichment culture procedures, by which these organisms can regularly be procured.

Familiarity with the results that can be achieved by means of enrichment cultures also leads to the conclusion that the distribution of diverse sorts of microbes is ubiquitous. For example, the cellulose decomposing *Cytophaga* and *Sporocytophaga* species, the nitrogen fixing azotobacters and *Clostridium pasteurianum*, or the hydrogen oxidizing bacteria that are known today can be isolated from soil, mud, or water samples in Argentina, Holland, Japan, Australia, Russia, or the U. S. with equal facility. And the pure cultures of one kind obtained in different places generally do not show any more differences among one another than do a variety of strains isolated in one and the same locality. A similar picture is presented by the microbes found in those "natural" enrichment cultures encountered in different parts of our globe, such as in hot springs, brine pools and salt beds, sulfur or iron springs. A careful comparison shows that, where the environmental conditions are closely comparable, the same types of organisms appear. The significance of these facts for determinative bacteriology is that we need not think in terms of local microfloras and -faunas. But they also carry another, less obvious implication.

There is now a large number of bacteria, yeasts, algae, and protozoa, incidentally observed under ill-defined conditions, that have been named and described on the basis of certain more or less easily ascertainable properties. Whether such characteristics, mostly determined by the application of stereotyped and arbitrary methods, bear any direct relation to those that are important in connection with the natural occurrence and survival of the organisms is often doubtful. I do not mean that it is totally useless to know that one type of photosynthetic bacteria can liquefy gelatin or grow in glucose media while others do not, or that certain fluorescent pseudomonads, in contrast to others, can utilize arabinose or produce acid from raffinose. But knowledge of such properties is entirely inadequate to tell us anything concerning the normal activities of the organisms in question. If the latter were better understood, it would become possible to approach the problems of determinative bacteriology and classification in a more rational manner, and to eliminate much of the present confusion.

For this purpose further studies with enrichment cultures are imperative. Beijerinck's great objective is still far from completed. It is necessary that conditions be more accurately controlled and specified, and that attention be paid to the effect on the outcome of enrichment cultures due to such variables as the reaction of the medium, the temperature of incubation, the concentrations of the various nutrient and non-nutrient ingredients, the presence or absence of micronutrients and growth factors, etc. It is very probable that by means of such refinements an ever increasing number of microörganisms will become accessible to isolation by enrichment culture techniques, and in this manner we shall learn more about the normal activities of the organisms encountered than by continued studies of pure cultures with standard methods. Of course, it must be admitted that such efforts may only slowly make it possible to recognize the natural environment of numerous organisms that have been isolated accidentally, and whose properties are now most imperfectly known.

When, in 1921, Beijerinck retired, a "Delft School" had been launched. The vast knowledge of the master had in part been transmitted to his students, and some of them continued the traditions in other Dutch institutions. Also outside the Netherlands his method of approach was spreading; men like Issatchenko and Krainsky, Melin, Gran, Krzemienievski, Kaserer, Stockhausen, and Stoklasa, who had worked in his laboratory, had gone back to their own countries and served as new nuclei abroad. And yet, when we think of a "Delft School" it is certainly not only these men who come to mind. Perhaps not even, in the first place, Beijerinck, but rather his successor, Albert Jan Kluyver, Corresponding Member of our Society of American Bacteriologists, the third of the great trio of Delft microbiologists, and the scientist in whose honor today's lecture is named. By developing the concept of comparative biochemistry Kluyver laid the foundation for an approach to biochemical problems that has proved to be one of the most fruitful of our era. It has brought order into a situation that was almost chaotic, and has become the guiding principle for the study of the chemical activities of any and all living organisms.

Two years after his inauguration Kluyver (13) made a survey of the processes known to occur in nature under the influence of microörganisms. It was a bewildering picture that emerged. Not only did it show the endless variety of substances, inorganic as well as organic, that can be decomposed by bacteria, molds, yeasts, etc.; it also illustrated the enormous diversity of substances that could arise during these decompositions. Now, awareness of diversity, a prerequisite for scientific pursuit, engenders the desire to discover unifying principles. Thus the problem arose: what common denominators can be found in this multiplicity of microbial activities?

The only one apparent in 1923 was the broadest possible generalization of Lavoisier's concept of biological oxidations as the source of energy for the maintenance of life. Pasteur had extended this idea by recognizing that fermentations, *i.e.* biological processes going on in the absence of air, are also energy yielding reactions. And Winogradsky, now some 60 years ago, had discovered organisms that could fulfill their energy requirements by oxidizing inorganic compounds. When computations of energy relations showed Kluyver that the multitude of known decompositions by microörganisms all proceeded with the liberation of energy it was, therefore, clear that Lavoisier's principle in this extended form could be invoked.

But this general answer did not satisfy Kluyver. It begged the question of a mechanism. After all, these decompositions could also be considered as chemical transformations, and since the beginning of the 19th century much constructive thought had gone into making chemical reactions intelligible on the basis of the atomic and molecular theories. That it might ultimately be possible to bring the comprehension of biochemical transformations up to the same level was, consequently, a reasonable expectation.

Besides, progress had been made in this direction. The researches of Neuberg on alcoholic fermentation by yeasts had achieved an interpretation of this process as the net result of a series of consecutive step reactions, each one chemically conceivable and simple in nature. Wieland had tackled the problem of the oxidation of alcohol to acetic acid by acetic acid bacteria and contended that this oxidation should be considered as composed of two stages, viz., the oxidation of alcohol to aldehyde, and of the latter—in the form of a hydrate—to acetic acid. Both these oxidations appeared to be reactions in which two hydrogen atoms are eliminated from a substrate molecule and transferred to any one of a number of hydrogen acceptors, such as $O_2$, quinone, methylene blue, etc. From these results Wieland had drawn the conclusion that all biological oxidations could be interpreted as primarily composed of series of dehydrogenations, with $O_2$ acting as the normal, but not the only possible H-acceptor. Harden and his coworkers, especially Grey, had made a good beginning with the resolution of the coli and aerobacter fermentations; and Fred and Peterson, as well as Speakman, of the butanol-acetone fermentation.

It would take too long to review the developments that led Kluyver, in a few years, to the masterly syntheses represented by the two major publications: "The unity in biochemistry" (14), and "The chemical activities of microörganisms" (15). It is in the latter treatise that the term "comparative biochemistry" was first used, and Kluyver envisaged for it an influence which could benefit biochemistry in a manner similar to that in which the concept of "comparative anatomy" had helped to bring order into the mass of isolated anatomical observations.

Kluyver's keen and critical mind recognized the potential significance of the ideas that Neuberg, Wieland, Warburg, Harden, and a few others had advanced to account for more or less specific biochemical events. Soon it became evident to him that those concepts could be welded together into a very few general principles, applicable to all biochemical phenomena. The most basic of these generalizations is the extension of the ideas of Neuberg and of Wieland to their ultimate limits. Thus, any biochemical process, whether oxidation, fermentation, or synthetic reaction, was considered as a chain of step reactions, each one of which represented a simple mechanism in which hydrogen is transferred from one molecule, the H-donor, to another, the H-acceptor. The only apparent exception to this principle was exhibited in the metabolism of complex molecules, composed of a number of simple entities, for example the polysaccharides (complexes of simple sugars), proteins (complexes of amino acids), and fats (complexes of fatty acids and glycerol). Such complexes would first be converted to their constituent units by hydrolytic cleavages, with the products subsequently undergoing the various hydrogen-transfer reactions. In this manner the existence of the many hydrolytic enzymes—glucosidases, proteinases, lipases, etc.—could be fitted into the general picture.

Many of the known facts concerning diverse metabolic processes could be readily incorporated into this concept. In the course of the following years numerous additional cases were investigated in his laboratory, and the outcome of this activity did much to strengthen the evidence for the soundness of the postulates. It also indicated that the initial stages in the biochemical transformations of a specific substance were very similar, if not identical, no matter what the final result proved to be. For example, the evidence strongly suggested the probability that in practically all instances of sugar decomposition the carbohydrate would first be degraded to three-carbon moieties; the differences in the end products reflected differences in the fate of these universal intermediate products. It is important to realize that the arguments applied to a great diversity of processes, such as the alcoholic and lactic acid fermentations, the "mixed acid" fermentation characteristic of the coli group, the butane-diol fermentation of *Aerobacter* and *Aerobacillus* species, the propionic acid fermentation, the butyric acid and the butanol fermentations, the fermentations in which acetone and isopropanol are produced. Also, many of the oxidative degradations appeared to proceed by the same initial stages.

It would be foolish to insist that the principles of comparative biochemistry would not have been developed if it had not been for Kluyver's penetrating approach, just as it would be foolish to contend that microörganisms would not have been discovered if Antonie van Leeuwenhoek had not done so. In the late twenties there were others who were beginning to think along similar lines, and the reconciliation of Wieland's and of Warburg's ideas on the nature of biological oxidations was proposed almost simultaneously by Kluyver and Donker, Szent-Györgi, and Fleisch, in three entirely independent publications. Nevertheless, the familiarity with the vast diversity of the conditions under which life can exist and manifest itself, especially in the world of microörganisms, made available for Kluyver's scientific contemplation an immensely greater range of patterns than that presented by the higher plants and animals. And the result was the enunciation of the most far-reaching generalization.

The attempts at interpreting various biochemical phenomena in greater detail led Kluyver and his collaborators to postulate a number of specific step reactions, leading to a small group of common intermediate products. It was clearly recognized that some substrates or intermediates could undergo more than one particular conversion. The extent to which each of the possible transformations occurs would, of course, depend on the nature of the organisms, *i.e.*, its enzymatic composition. But even for the same organism the result is usually not fixed because environmental conditions, such as temperature, concentration of substrate or intermediate products, reaction of the medium, the presence or absence of special hydrogen donors or acceptors, could readily influence the magnitude of the different conversions. It is, therefore, impossible to predict the exact outcome of a biochemical process in terms of the precise quantities in which each of the end products will be formed. The frequently observed fluctuations in this respect need not be disturbing, however; they become readily understandable as the result of a complicated interplay between the various potentially possible reactions in which the intermediate products can participate. When viewed in this manner a biochemical reaction becomes more clearly a dynamic event, to be represented by series of steps with variations in several directions rather than by a single chemical equation with fixed quantitative relations between the end products.

Many of the step reactions and intermediate products postulated by Kluyver some twenty years ago appear outmoded to-day. Surely, no biochemist would now seriously consider methyl glyoxal, for example, in the central position which

Kluyver assigned to it in his London Lectures (15). Much has been accomplished in the intervening years through the brilliant work of many scientists. The chemical nature of several intermediate products has been established with increasing precision; the interactions and conversions of these compounds can now be represented by reaction chains far more elaborate than was once deemed possible. In large part this astounding penetration into details of biochemical mechanisms has resulted from the isolation of specific enzymes with which partial conversions can be investigated under rigorously defined conditions. And much of this work has been done with microörganisms; those who have attended the symposium on the first day of our meetings will realize this.

Furthermore, new principles have been introduced. Among the most important ones must be mentioned Michaelis' theory (16) of the single-electron shifts; Lipmann's concept (17) of the high-energy phosphate bond and its significance for the preservation and storage of energy; and the ideas concerning the transfer of whole blocks of atoms, as in trans-aminations, trans-methylations, trans-acetylations, trans-glucosidations and trans-phosphorylations. It has been a phenomenal development. But, although these advances have shown the need for modifying the earlier postulated details, they have also served to substantiate the validity of Kluyver's main thesis regarding the fundamental unity in biochemistry. The basic similarity in the biochemical behavior of so many different organisms is now generally admitted. It is emphasized by the occurrence of the same amino acids, vitamins, enzymes, etc., in all forms of life, and by the participation of a number of identical intermediate products in practically all metabolic activities.

The recognition of this unity is Kluyver's great contribution; it is also the starting point of "comparative biochemistry". Predicated upon the fact that a particular substance, whether substrate or intermediate product, can undergo only a limited number of immediate transformations, sometimes only a single one, these can be explored by investigating the fate of such compounds under the influence of different organisms. The results so far obtained have amply demonstrated the fruitfulness of this line of study.

A good example is furnished by the methane fermentation, a process in which various alcohols and fatty acids are decomposed to methane, generally accompanied by the production of carbon dioxide. Now, the primary attack on those substrates cannot readily be conceived of as anything but a straight dehydrogenation. Hence an external hydrogen acceptor is required. Decompositions of the same substrates are known to occur in the presence of oxygen, nitrate, and sulfate, and these substances, acting as hydrogen acceptors, are thus converted into $H_2O$, $H_3N$, or $H_2S$ respectively. This led to the idea that the methane fermentation represents a similar substrate oxidation with $CO_2$ as hydrogen acceptor, a postulate for which the investigations of Barker (19) have furnished experimental evidence. The degradation of the higher fatty acids during the methane fermentation has been shown by Mrs. Stadtman (personal communication) to follow exactly the path required by the Knoop-Dakin theory for this process in higher animals. Hence the methane fermentation no longer occupies a totally unique position. The details of the mechanism whereby carbon dioxide is reduced to methane remain to be elucidated, and these might yield important results for an understanding of the mechanism of photosynthesis.

I realize that this may seem a far-fetched conclusion. However, the following considerations, in exposing the trend of thought upon which this deduction rests, should make it appear reasonable. A comparison between the photosynthetic activities of green plants and of green and purple bacteria suggested, several years ago, that photosynthesis should be interpreted as a process of carbon dioxide reduction with hydrogen obtained by a photochemical decomposition of water (20). This, in turn, implies that the reactions more immediately concerned with the assimilation and reduction of carbon dioxide must themselves be non-photochemical processes. It should consequently be possible to reach a better understanding of the essential features of these reactions by a comparative study of all cases in which carbon dioxide is similarly involved. And those include not only the carbon dioxide assimilation by chemo-autotrophic microbes, but also the Wood and Werkman reaction (21), the formation of other di- and tricarboxylic acids by carbon dioxide addition to various keto-compounds (22), the production of acetic acid from $CO_2$ and hydrogen by *Clostridium aceticum* (23), and the methane fermentation. Such a comparative study would make it possible to discover the common denominators of all these processes, and therefore contribute to a more detailed picture of the photosynthetic reaction.

Two decades ago Kluyver advocated the use of microörganisms for comparative biochemical studies. On several occasions he stressed the advantages they offered, both on account of the ease of handling them under controlled and reproducible conditions, and because of the enormous biochemical versatility encountered within this group. It is often possible to select a specific microörganism as singularly appropriate for a given problem because it carries out a certain type of reaction to the exclusion of almost any other. But it is equally important to realize that one may find among these creatures the best examples of seemingly quite different biochemical properties with respect to the conversion of a particular substrate. Both of these aspects are important for a comparative biochemical approach. If it be further remembered that by the application of Beijerinck's principle of enrichment cultures many of the organisms are so readily procurable, it will be clear that the case for the microbes—and for the microbiologist—is pretty strong.

This has obviously been recognized. During the past decade there has been a rapidly growing interest in comparative biochemistry as well as in microörganisms. It is no longer unusual to find a large fraction of the pages of physiological and biochemical journals occupied by publications dealing with the activities of fungi, protozoa, and bacteria. Even in the field of genetics the mold Neurospora, the yeasts, *Escherichia coli*, Paramecium, and bacteriophages are successfully competing with Oenothera, *Zea mais*, and Drosophila.

When nowadays enzyme reactions are studied by methods ranging from kinetic measurements (24, 25) to the use of mashed cells, of dried cell preparations (26), of cultures supplied with sub-optimal amounts of growth factors (27–30), of induced mutations (31), of anti-vitamins (32), or of adaptive enzyme systems (33), one finds that microörganisms are used in the majority of cases. But it must be realized that this is generally done with the tacit implications that the results will be of importance for a better understanding also of similar processes occurring in other organisms. This attitude has been amply justified. The earlier remarks concerning the mode of degradation of higher fatty acids in the methane fermentation and in the animal body provide a good example. The recent studies of Heidelberger *et al.* (34, 35) on the decomposition of tryptophan in mammals have shown that this is accomplished by a mechanism which appears to be identical with that previously demonstrated in the mold Neurospora. It will be superfluous to elaborate this theme any further.

The increased interest in general microbiology is apparent also in other ways. It is no longer necessary for the confirmed microbiologist to feel that he supports a worthy cause in vain when he expresses the devout wish that those responsible for the development of science in colleges and universities might eventually "see the light", and establish positions for teaching and research in this field. Surely, general microbiology is now a rapidly expanding science, and I firmly believe that it is an easily defensible thesis to propose that its spectacular rise is due in large part to the Delft School. However, the encouragement by our institutions of higher learning might also have another cause. Some of the interest displayed could have been stirred up by an overemphasis on developments of the past decade resulting from studies on vitamins, chemotherapy, and antibiotics. These researches have been well publicized, and rightly so, because the advances made have been striking, and the applications both numerous and successful. Nevertheless, these topics represent only a small segment of the field of general microbiology, and I hope that the other aspects will not be neglected.

That Albert Jan Kluyver was chosen as the microbiologist who is to be honored to-day bears convincing witness to the fact that our Society of American Bacteriologists is concerned with the broad principles. For the work of the "Delft School" carries implications of deep philosophical importance that must appeal to any one who is still willing to subscribe to Ernest Renan's dictum: "Le but du monde, c'est l'Idée."

Beijerinck's major contributions can be considered as the first direct experimental investigations of Darwin's principle of natural selection. In the enrichment cultures the experimentally defined environmental conditions are the selecting agent, and the outcome of the cultures can provide an unambiguous answer to the question as to what organisms among the many types present in the inoculum are most fit to cope with the environment. This having been established by the "endless experiments", one can even try to penetrate further, and determine the mechanism by which the selection operates.

So far, it can be stated with some assurance that the significant factors are physical (light, temperature, concentrations) and chemical in nature. In many cases it is obvious that those organisms whose minimum nutrient requirements are fulfilled by the culture medium will come to the fore. This, together with other cases of successful competition, operating through the production by one kind of organism of substances which inhibit or prevent the development of other competitors, constitutes strong evidence in support of the idea that ecology, at least as far as microörganisms are concerned, rests principally on a biochemical basis.

The significance of biochemistry for a better understanding of the behavior of living organisms is further attested to by the modern trends in genetics. Nearly all the studies in this field of physiological or biochemical genetics are carried out with microbes, and most of these investigations are patterned on the important work of Beadle and Tatum and their collaborators. What has come out of the numerous contributions, in which algae, molds, yeasts, protozoa, bacteria, and phage play so important a part, supports an idea expressed as early as 1917 by Beijerinck (36), namely, that genetic characters function by way of controlling the formation of enzymes. There is now a wealth of information in favor of the supposition that one genetic character is involved in the control of a single enzyme. A by-product of these studies has been the use of genetically modified strains for the successful elucidation of the detailed mechanism of biochemical syntheses.

But there are also many examples known in which environmental rather than genetic factors influence directly the enzymatic composition of microörganisms. These are, of course, the numerous cases of adaptive enzyme formation in which the presence of a particular substrate elicits the formation of a corresponding enzyme system capable of catalyzing the transformation of the substrate in question. It is possible to submit that in such instances the genetic constitution of the organism confers upon it the potentiality of responding to an environmental stimulus. However, the common interpretation of the one gene-one enzyme concept does not generally connote such a degree of flexibility, and it will be interesting to see how the ideas on the fundamentals of genetics will, in the course of time, be modified so that they can account for the determinative effect of external factors.

These phenomena of adaptation bring into sharp focus the fact that microbes, like human beings, are subject to and respond to environmental influences, and do not necessarily represent rigidly determined systems. This adaptability, together with the frequent spontaneous mutations exhibited by living organisms, emphasizes their innate variability. In another, perhaps more basic sense, there is no denying the existence of a high degree of constancy. These two aspects of life—its constancy and variability—are reflected in many ways. From the point of view of comparative biochemistry, the constancy finds its expression and counterpart in the unity of the fundamental biochemical mechanisms, that is, Kluyver's concept of the "unity in biochemistry." This, to-day, is also the most compelling argument in favor of a monophyletic origin of life. The variability, by comparison, can be related to the existing biochemical diversity, so glaringly apparent especially among microörganisms, and it represents the numerous directions in which adaptations to a new environment have become established. The persistence of so many patterns, like variations of a theme, drives home the importance of individuality, without which there could be no differences—nor evolution.

And I hope that you may be found willing to consider seriously the proposition that an important aspect of evolution consists in the acquisition of increased comprehension. Comprehension not for the sake of power—there is too much of that in the hands of too few—but for the sake of a possible evolution of man to a state in which he is no longer at war with himself and his contemporaries, no longer at odds with nature, but an integral part of it. The implication of this is the need for recognition of the intrinsic value of the individual as the unique, potential step towards something new and better. If this is appreciated we shall also have gone far in understanding the great significance of another phase of the profound influence wielded by the founder of the "Delft School". For Albert Jan Kluyver has been a living example of this attitude towards the individual. Those who have had the great good fortune of experiencing his influence—and there are many of them among my audience—can never be grateful enough.

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